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Evaluation of the Sysmex XT-2000iV hematology instrument for use with feline blood

Inaugural-Dissertation

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In Dankbarkeit meinen Eltern gewidmet,
die mir meine Ausbildung ermöglicht haben,
und Wanda, die mich immer unterstützt hat.

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Index of abbreviations

WBC	White blood cell
NEU	Neutrophil granulocyte
LYM	Lymphocyte
MONO	Monocyte
EOS	Eosinophil granulocyte
BASO	Basophil granulocyte
RBC	Red blood cell
HGB	Hemoglobin
HCT	Hematocrit
MCV	Mean corpuscular volume
MCH	Mean corpuscular hemoglobin
MCHC	Mean corpuscular hemoglobin concentration
RET	Reticulocytes
LFR	Low fluorescence ratio of reticulocytes
MFR	Middle fluorescence ratio of reticulocytes
HFR	High fluorescence ratio of reticulocytes
IRF	Immature reticulocyte fraction
PLT-O	Platelets measured optically
PLT-I	Platelets measured with impedance

Abstract

The objective of this study was to evaluate the Sysmex XT-2000iV with respect to its use with samples from the feline species.
The Sysmex XT-2000iV is a flow cytometer that analyzes 31 hematological parameters and is based on the principles of multi-angle polarized light scatter separation for WBC, WBC differential, RET and PLT-O. In addition, the impedance method is used to determine the parameters RBC and PLT-I, and SLS (sodium lauryl sulfate) is used for HGB.
The reference methods used were those recommended by the International Committee for Standardization in Haematology (ICSH). These included centrifugal hematocrit, the CellDyn® 3500 analyzer for RBC, red cell indices, and WBC, microscopic differentiation of 200 WBC (100 cells each by two technicians), and manual RET examination.
The coefficients of variation for precision in series were good to excellent and for precision from day to day excellent.
The tested range of linearity extended the reference ranges up to $100x10^3/\mu l$ for WBC, $19.6x10^6/\mu l$ for RBC, 29.1 g/dl for HGB, 82% for HCT, $98.2x10^3/\mu l$ for RET, and $4140x10^3/\mu l$ for PLT.
The correlations between the Sysmex XT-2000iV and the reference methods were good to excellent, except for MONO# ($r = 0.65$).
Additionally, quantitation of RET allowed characterization of regenerative versus non-regenerative anemias.
From this evaluation, we conclude that the Sysmex XT-2000iV is an excellent and highly reliable hematology instrument for the analysis of feline blood samples.

1 Introduction

Hematological parameters provide important information on a patient's state of health, disease history or response to therapy. Most automated cell counters were developed for human blood samples. The Sysmex XT-2000iV is one of few that have been designed to analyze blood samples from different species. The instrument is equipped with hardware and software features allowing the analysis of blood samples from the rat, mouse, guinea pig, rabbit, monkey, dog, cat and horse. The software also allows the definition and storage of instrument factors, optimized by the user, to analyze blood samples of additional species.

2 Materials and Methods

2.1 Study Design

This evaluation was based on the analysis of 409 blood samples, collected by venipuncture, from cats that presented at the Clinic for Small Animal Medicine and Clinic for Small Animal Surgery of the Vetsuisse Faculty, University of Zurich. The samples were used to assess the precision, linearity and accuracy of the instrument. Samples were collected in tubes containing K_3-EDTA. After arrival at the laboratory, the samples were simultaneously analyzed using reference methods and the Sysmex XT. All samples were analyzed within two hours after collection, except during those experiments designed to study the effect of aging on the cells. The goals of this study were:

1. To evaluate the overall performance of the Sysmex XT-2000iV on cat blood
2. To determine the accuracy of the instrument by comparing the results with those from established reference methods
3. To determine the instrument precision (in series, as well as from day to day)
4. To evaluate linearity

2.2 Sysmex XT-2000iV System

The Sysmex XT-2000iV is a fully automated hematology analyzer for animal blood, which can evaluate 31 parameters of a blood sample: WBC count, RBC count, HGB, HCT, MCV, MCH, MCHC, PLT-I, PLT-O, RDW-CV (coefficient of variation of red cell distribution width), RDW-SD (standard deviation of red cell distribution width), MPV (mean platelet volume), PDW (distribution width of platelets), P-LCR (platelet-large cell ratio), PCT (plateletcrit), NEU (neutrophil granulocyte count and percentage), LYM (lymphocyte count and percentage), MONO (monocyte count and percentage), EOS (eosinophil granulocyte count and percentage), BASO (basophil granulocyte count and percentage), RET (reticulocyte count and percentage), LFR, MFR, HFR, and IRF.
WBC and reticulocytes are analyzed via a fluorescence flow cytometry method, using a semiconductor laser. When the laser hits a cell, the light is scattered in all directions. This light contains all information about the physical properties of a cell (such as size and structure). This scattered light is detected by photodiodes, or a photomultiplier in the case of fluorescence, and is then converted into electrical pulses. RBC and platelet counts are analyzed via the impedance method, using hydrodynamic focusing (Rodriguez-Trujillo, Castillo-Fernandez *et al*. 2008), which focuses the cells, forcing

them to pass one-by-one through the light beam. After passing through the aperture, the diluted sample is sent to the catcher tube. This prevents the blood cells behind the orifice from drifting back or causing abnormal blood cell pulses. Hemoglobin is analyzed photometrically, at a wavelength of 555 nm, based on the SLS (sodium lauryl sulfate) hemoglobin detection method (Oshiro, Takenaka *et al*. 1982; Evans and Smith 1992).
With its capacity to analyze 80 samples per hour, and with only short daily maintenance work required, the Sysmex XT is highly effective, allowing the analysis of a large number of samples per day. The autosampler, as well as the handling of the IPU (Information Processing Unit), where the analyzed data is displayed, provide for ease of use. The accuracy of the analyses is ensured by an internal quality control that detects and eliminates variations.

2.3 Reference methods

The CellDyn 3500 was used as the reference instrument (Sanzari, De Toni *et al*. 1998; Kieffer, Winkler *et al*. 1999). This hematology analyzer is based on the combination of the impedance method, pioneered by Coulter (Coulter 1956), and flow cytometry (Fulwyler 1974). White blood cells are counted by two separate channels, the electrical impedance channel (white blood cell impedance count = WIC) and the optical flow channel (white blood cell optical count = WOC). Differentiation of WBCs is done in the WOC channel. The hemoglobin concentration is measured spectrophotometrically, on the basis of a hemiglobinhydroxylamine method. The indices of the red blood cells and platelets are calculated. The following parameters generated by the CellDyn 3500 were used as reference values: WBC, RBC, HGB, MCV, MCH, and MCHC.
Microscopic methods were used to determine WBC differential and RET and PLT counts. For the WBC differential, blood smears were stained using an automated staining instrument. Two technicians, each with more than ten years of experience in veterinary hematology, differentiated 100 cells per smear. The mean of the 200 cells was used to calculate the percentage distribution of the WBC differential. Absolute values of leukocyte differentials were obtained by multiplying the absolute WBC count from the CellDyn 3500 by the microscopically determined percentage of each WBC subpopulation, including neutrophils, lymphocytes, monocytes, eosinophils and basophils. Manual RET counts were performed by enumerating the RET as a percentage of 1000 mature RBC, using a standard method based on Brilliant Cresyl Blue (Cossandi and Maggiora 1952) stained blood smears. Only the aggregated reticulocytes, which represent the more immature cells and are characterized by large clumps or strands of precipitated nucleoprotein (Perkins, Grindem *et al*. 1995), were counted. Absolute values of reticulocytes were calculated by multiplying the microscopically determined fraction of RET by the RBC count from the CellDyn 3500.
The PLT counts were determined using a Neubauer hemocytometer. Manual counting was necessary, as feline platelets cannot be determined using the Coulter principle, because large platelets overlap in volume with small erythrocytes. The blood was diluted with a Calibra® digital pipette at a ratio of 1:100 in ThromboCount pur, which is an oxalate-buffered solution that leads to a disaggregation and rounding of the PLT and lysis of the erythrocytes, but not of the PLT, WBC or RET. PLT were microscopically counted by two technicians with similar experience in veterinary hematology as those mentioned above. The mean of the two counts was used as the reference value.

2.4 Precision

The precision within series was evaluated according to the methods published by Knoll and Rowell . The precision of the Sysmex XT-2000iV was determined on the basis of 12 analyses of fresh, K_3-EDTA-anticoagulated blood samples. The blood samples were collected from cats with high, intermediate and low values for each parameter. For these measurements, 1.5-2 ml of blood was used. The blood samples were stored at room temperature during the analysis.
Precision was assessed for the counting of WBC, NEU (# and %), LYM (# and %), MONO (# and %), EOS (# and %), BASO (# and %), RBC, RET, LFR, MFR, HFR, IRF, and PLT, and for the measurements of HGB, HCT, MCV, MCH, MCHC, RDW-SD, RDW-CV.
Precision from day to day was measured with three commercial control blood samples (e-CHECK Level 1, 2 and 3), provided by Sysmex, which were analyzed once daily during a 12-day period.

2.5 Linearity

The linearity of the measurements was established to determine whether blood values outside of the reference range could be measured accurately. It is important to determine the range of linearity, especially for elevated cell counts. These counts can potentially be underestimated because, with an increasing number of cells, the probability that multiple cells will pass through the laser beam or the impedance orifice simultaneously increases. This phenomenon is known as coincidence (Strackee 1966). The linearity of the measurement range was determined using three K_3-EDTA anticoagulated feline blood samples. They were used to determine the linearity of the measurement range for WBC, LYM, MONO, NEU, EOS, HCT, RBC, HGB, RET, and PLT. In order to get hematological values above and below the reference range, the blood samples were centrifuged at 390×g for five to ten minutes. The plasma was then removed from the blood cells. The concentrated blood cells were diluted with autologous plasma, in steps of 10%, to obtain a dilution series from 0% (plasma alone) up to 100% blood cell concentrate (undiluted blood cell concentrate). The measured values were plotted on an x-y graph, and the correlation coefficient and regression were calculated using the least squares method (Bablok 1985). The equation of the resulting line was described as $y = a + bx$. The range of linearity was determined by visual inspection of the plots.

2.6 Accuracy

The accuracy was determined by comparison of the results obtained from the Sysmex XT-2000iV with those from the reference methods for a total of 409 feline blood samples. The linear regression lines and correlation coefficients were determined according to Passing and Bablok (Bablok 1985).

2.7 Evaluation of the clinical relevance of results obtained from the Sysmex XT-2000iV

For each sample, the data from the Sysmex XT and CellDyn were compared with established hematology reference values for cats (Table 1). These values were determined previously in the clinical laboratory, using the CellDyn 3500 instrument (Kieffer, Winkler *et al*. 1999) and manual methods. The results were judged to be below, within or above the reference range, and the resulting interpretations from the Sysmex XT and the reference methods were compiled and compared to each other. If a Sysmex XT result suggested a different clinical conclusion than that made based on the reference method, the result was judged with respect to the degree of the deviation. Two categories were defined, minor and major discrepancies. Minor deviations were defined as being <10% different from the lower or upper reference value. Major deviations were defined as discrepancies of more than 10%, which would have led to severe clinical misinterpretation. One example of a major deviation was a sample where the reference method generated a result of 370 lymphocytes per µl, and the Sysmex XT yielded 4250/ µl, which is >10% of the upper reference value.

Table 1 Reference values of haematological parameters for cats used in this study

Parameter	Value
RBC (10^6/µl)	7-10.7
HGB (g/dl)	11.3-15.5
HCT (%)	33-45
MCV (fl)	41-49
MCHC (g/dl)	33-36
RET (/µl)	-60'000
RET (%)	-1
PLT (10^3/µl)	180-680
WBC (10^3/µl)	4.6-12.8
LYM (10^3/µl)	1.05-6
MONO (10^3/µl)	0.046-0.678
NEU (10^3/µl)	2.315-10.134
EOS (10^3/µl)	0.1-0.6

2.8 Message codes

IP (interpretive program) messages, also called flags, are displayed by the IPU when the instrument judges that, based on comprehensive surveys of numerical data and particle size distribution scattergrams predetermined by the manufacturer, something about the sample is flawed. Reasons for flags might include cells that cannot be assigned to a population or values that exceed certain limits. The purpose of these messages is to inform the user about the abnormality, so that special measurements or further analysis can be conducted.

2.9 Aging of the cells

To study the effect of K_3-EDTA treatment on blood cell aging, blood was collected from seven cats, stored at room temperature, and analyzed immediately after collection and after 1, 2, 3, 4, 5, 6, 7, 8 and 24 hours.

2.10 Statistical methods

All data were stored using the program Excel and analyzed by Analyse It for Excel. Precision was determined by calculation of the coefficients of variation (CV).

3 Results

3.1 Display of measured values

After each measurement, the results of the analysis are shown on the display of the IPU, together with a graphical display of the reference ranges, as well as dot plots in the form of scattergrams and histograms. Cell populations are represented by colored clouds – neutrophils were marked light blue, lymphocytes purple, monocytes green, eosinophils red, and cell trash dark blue. The correct differentiation of the cells can be estimated visually, and an experienced user can use these scatter- and histograms to obtain further information about the blood samples. For example, a left shift in the neutrophil granulocytes can be recognized from the scattergram as an extension, or even a fusion, from the mature granulocyte population towards the lymphocyte and/or monocyte population. If these populations are fused, the instrument cannot correctly differentiate the populations, and the message code "WBC abnormal scattergram" is displayed. The fused cell cloud will be gray. This helps the user to identify those samples that have to be differentiated manually. In 17 out of 70 analyzed blood samples with a left shift, the scattergram showed this phenomenon of fused clouds; in the others, a shift to the upper left was visible. Scattergrams are shown for a WBC Diff in Fig 1a, a RET in Fig 1b, a PLT-O in Fig 1c, and a left shift of neutrophils in Fig 1d. Histograms for RBC and PLT-I are shown in Fig 1e and Fig 1f, respectively. The results could be printed out or saved on the IPU.

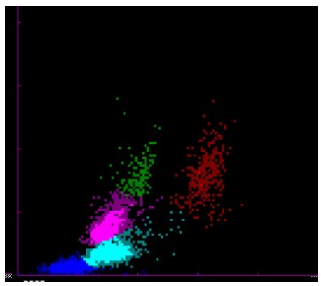


Fig 1a Scattergram of WBC Diff

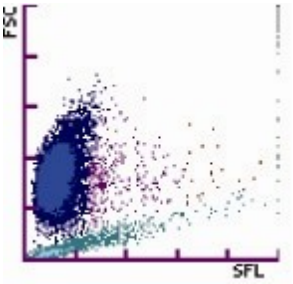


Fig 1b Scattergram of RET

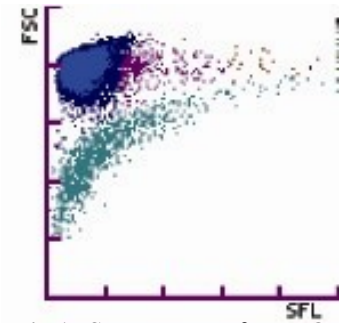


Fig 1c Scattergram of PLT-O

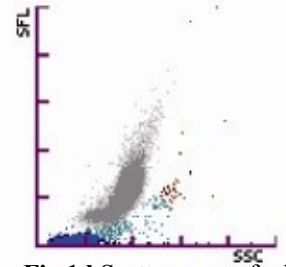


Fig 1d Scattergram of a left shift of neutrophils

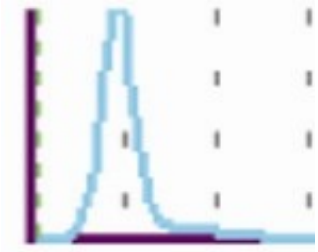


Fig 1e Histogram RBC

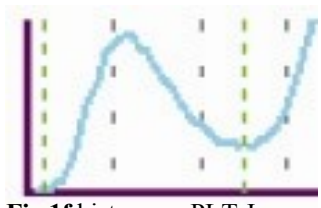


Fig 1f histogram PLT-I

3.2 Precision

The mean values and coefficients of variation for precision in series are summarized in Table 2. Precision from day to day was measured with three control blood samples (e-CHECK Level 1, 2 and 3), which were analyzed once daily during a 12-day period. From these results, mean and coefficient of variation were calculated. The results are displayed in Table 3.

Table 2 Precision in series: mean values and coefficients of variation for blood samples with either low, middle or high values

Parameter	Low		Middle		High	
	Mean	CV %	Mean	CV %	Mean	CV %
WBC $x10^3/\mu l$	1.84	1.98	11.38	1.14	41.83	0.92
NEU $x10^3/\mu l$	0.97	3.55	8.78	1.52	27.77	2.56
NEU %	52.67	2.34	77.11	0.56	66.36	2.67
LYM $x10^3/\mu l$	0.60	4.66	1.95	1.96	9.81	8.16
LYM %	32.42	4.32	17.14	2.10	23.45	7.77
MONO $x10^3/\mu l$	0.05	21.23	0.48	3.95	2.45	4.04
MONO %	2.98	21.76	4.20	5.08	5.87	4.20
EOS $x10^3/\mu l$	0.22	10.01	0.17	9.90	1.58	5.03
EOS %	11.92	10.39	1.47	10.62	3.77	5.07
BASO $x10^3/\mu l$	0	0	0.01	26.65	0.22	9.42
BASO %	0	0	0.11	26.65	0.55	9.50
RBC $x10^6/\mu l$	4.26	1.06	8.07	0.46	16.49	0.41
HGB g/dl	5.2	0	13.06	0.69	21.39	0.32
HCT %	16.1	0.88	36.79	0.65	53.37	0.28
MCV fl	37.81	0.35	45.56	0.76	32.37	0.27
MCH pg	12.21	1.18	16.16	0.67	12.97	0.48
MCHC g/dl	32.3	0.88	35.49	1.28	40.08	0.41
RDW-SD fl	24.28	0.52	28.57	0.74	33.57	0.56
RDW-CV %	18.69	0.42	20.14	0.93	34.48	0.56
RET $x10^3/\mu l$	33.08	6.36			206.25	6.81
RET %	0.78	6.44			2.52	6.8
LFR %	95.69	2.11			89.31	3.28
MFR %	3.44	44.19			8.76	28.72
HFR %	0.87	88.06			1.93	30.41
IRF %	4.31	46.87			10.69	27.41
PLT-O $x10^3/\mu l$	143.92	4.57	350.42	2.48	759.83	1.35
PLT-I $x10^3/\mu l$	107.92	39.44	172	43.63	758.58	1.92

Table 3 Precision from day to day: mean value and coefficient of variation for control blood samples

Parameter	Level 1		Level 2		Level 3	
	Mean	CV	Mean	CV	Mean	CV
WBC (10^3/µl)	3.07	2.05%	7.07	1.40%	16.43	0.85%
RBC (10^6/uL)	2.39	0.71%	4.45	0.54%	5.46	0.57%
HGB (g/dL)	5.9	0.85%	12.3	1.22%	17	0.59%
HCT (%)	18.1	0.88%	36.5	0.60%	49.6	0.58%
MCV (fL)	75.7	0.45%	82	0.41%	91	0.40%
MCH (pg)	24.6	1.10%	27.7	1.23%	31.2	0.87%
MCHC (g/dL)	32.5	1.23%	33.8	1.21%	34.3	0.76%
PLT-I (10^3/uL)	58.3	3.09%	221	2.90%	510	1.88%
PLT-O (10^3/uL)	58.2	3.78%	199	2.21%	477	1.82%
RDW-SD (fL)	44	0.70%	44	0.59%	44.6	0.65%
RDW-CV (%)	16.3	0.61%	15.1	0.33%	14	0.43%
PDW	75.3	3.59%	77.3	1.68%	81.1	1.48%
MPV (fL)	8.5	2.35%	8.8	0.91%	8.9	0.56%
P-LCR (%)	9.8	7.86%	9.9	3.85%	10.5	2.76%
PCT (%)	0.05	18.95%	0.2	3.59%	0.46	2.63%
NEU# (10^3/µl)	1.37	2.63%	3.38	2.37%	8.71	1.07%
NEU% (%)	44.7	1.97%	47.7	1.68%	53	0.96%
LYM# (10^3/µl)	1.04	5.48%	2.16	4.17%	3.96	3.03%
LYM% (%)	33.9	4.72%	30.5	3.61%	24.1	2.78%
MONO# (10^3/µl)	0.37	9.65%	0.83	7.85%	1.96	5.05%
MONO% (%)	12.2	9.84%	11.7	8.55%	12	5.25%
EO# (10^3/µl)	0.28	7.42%	0.71	7.08%	1.8	5.11%
EO% (%)	9.2	6.62%	9.9	6.91%	11	4.81%
BASO# (10^3/µl)	1.95	3.23%	4.67	2.03%	12.35	0.89%
BASO% (%)	63.4	1.89%	66	1.18%	75.2	0.78%
RET# (10^3/µl)	175	2.92%	119	3.61%	53	4.91%
RET% (%)	7.3	2.74%	2.68	3.73%	0.97	4.74%
LFR (%)	70.8	3.11%	73.6	3.40%	80	2.75%
MFR (%)	24.3	7.00%	21.6	9.72%	16.5	12.73%
HFR (%)	4.9	12.88%	4.8	29.29%	3.5	37.14%

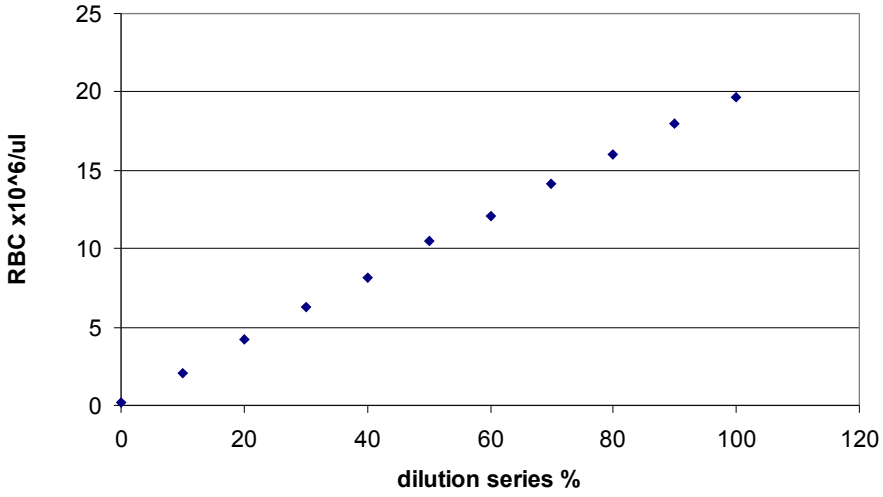
3.3 Linearity

Table 4 shows the parameters for which linearity was tested. Fig 2 shows the corresponding graph for RBC. For monocytes and basophils, linearity was not evaluated because the cell numbers were too low. By visual inspection of the graphs, it was found that, for RBC, HGB, HCT, NEU, LYM, EOS and PLT, linearity was maintained within the tested ranges. For WBC, linearity was maintained up to 100x10^3/ µl, and for RET, up to 46.6 x 10^3/µl, with a good correlation.

Table 4 Linearity of the measurement range of blood samples from cats with coefficient of correlation, regression line and the range of the tested parameters

Parameter	Coefficient of correlation r	Range of linearity	Reference range according to the Sysmex XT
WBC	0.996	0.05-100 x 10^3/µl	0-310 x 10^3/µl
NEU	0.996	0-5.03 x 10^3/µl	
LYM	0.994	0-1.62 x 10^3/µl	
EOS	0.986	0-0.37 x 10^3/µl	
RBC	0.999	0.18-19.6 x10^6/µl	0-14 x 10^6/µl
HGB	0.999	0-29.1 g/dl	0-25 g/dl
HCT	0.999	0-82%	0-70 %
RET	0.964	0-98.2 x 10^3/µl	0-3 x 10^6/µl
PLT	0.99	0-4140 x 10^3/µl	0-2000 x 10^3/µl

Fig 2 Linearity of the measurement range for RBC over a range of 0.18-19.6 10^6/µl. X-axis: dilution series; Y-axis: RBC values measured by the Sysmex XT



3.4 Accuracy

The results of the comparison of the methods are compiled in Table 5. As an example, the agreement of the RBC values is graphically displayed in Fig 3. The Sysmex instrument displayed the platelet indices MPV, PCT and PDW only when clear separation of RBC and platelets was possible, which rarely occurred. In view of the relatively low clinical significance of the platelet indices, these parameters were excluded from further evaluation in this study. Visual inspection of the results revealed values that were clearly apart from the regression line and were, therefore, considered "outliers." A total of 22 outliers were identified for WBC (2), HGB (1), HCT (6), NEU (1), MONO (4) and BASO (1), PLT (2) and RET (5).
In cases where the Sysmex XT was not able to differentiate between at least two cell types, it did not display the results for those cells in the normal display mode, but in the research mode. In the scattergram, cells that could not clearly be assigned to a given population were thus shown in gray. The Sysmex XT indicated that such a sample

should be differentiated manually. When these samples with unassigned cells were removed from the evaluation, the correlation coefficient increased considerably: NEU 0.94 to 0.98; LYM 0.45 to 0.92; MONO 0.2 to 0.65; EOS no change, BASO no research data.
The rather low values for the correlation coefficients for MONO, EOS and LYMPH could be due to the poor precision in series of the manual differential (Dutcher 1984), which is shown in Table 6. The bad correlation between the manual reticulocyte counting and the results obtained with the Sysmex XT is not unexpected, as manual microscopic reticulocyte counting is known to be imprecise and unreliable (Peebles, Hochberg *et al*. 1981; Savage, Skoog *et al*. 1985). The coefficient of variation for the manual reticulocyte count was 31.4% for high and 36.7% for low RET.
In the determination of PLT, the Sysmex XT tended to underestimate the number obtained by the manual count. This can be seen in the linear regression equation
(y = -0.89 +0.96x), but this result is of no further concern.

Table 5 Accuracy of the Sysmex XT-2000iV, determined by comparison of the results with those of the reference methods

Parameter	Sample number n	Coefficient of correlation r	Intercept a	Slope b	Significance of differences of methods[a]
WBC	409	0.98	-0.07	1.05	<0.0001
NEU #	193	0.98	0.16	0.98	0.9277
LYM #	193	0.92	0.1	1.12	<0.0001
MONO #	201	0.65	0.02	1.42	<0.0001
EOS #	208	0.87	0.04	0.97	0.002
BASO #	216	-0.03	0.0	0.57	0.4391
RBC	409	0.99	-0.05	1.01	0.17
HGB	409	0.99	-0.87	1.08	0.4414
HCT	409	0.97	-1.35	1.05	0.001
MCV	409	0.95	-14.88	1.32	<0.0001
MCH	409	0.95	0.58	0.96	0.0004
MCHC	409	0.42	-68.58	3.06	<0.0001
RET #	100	0.73	14606	1.52	<0.0001
RET %	100	0.85	0.48	1.35	<0.0001
IRF #	100	0.75	1323	0.28	<0.0001
IRF %	100	0.85	0.02	0.29	<0.0001
PLT	100	0.95	-0.89	0.96	0.0683

[a] Significance of differences of methods evaluated by the Wilcoxon matched-pairs signed-ranks test

Fig 3 Comparison of RBC counts determined by CellDyn 3500 (X-axis) and Sysmex XT (Y-axis). The linear regression line and coefficient of correlation were calculated according to Passing and Bablock; n = 409, r = 0.99, y = 0.0042+1.001x

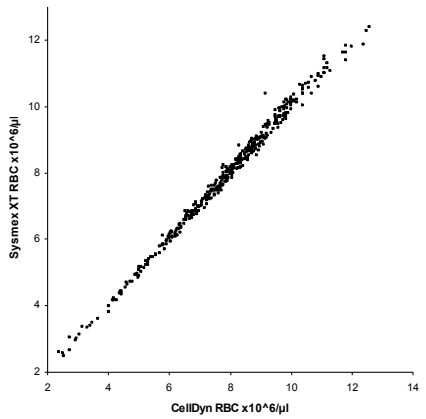


Table 6 Precision in series of the manual differential

	Mean (%)	S	CV
STAB NEU	0.4167	0.6686	160.45 %
SEG NEU	77.6667	4.6775	6.02 %
LYM	15.5833	3.5792	22.97 %
MONO	2.5833	1.9287	74.66 %
EOS	3.75	1.9129	51.01 %
BASO	0	0	0 %

3.5 Evaluation of the clinical relevance of results obtained with the Sysmex XT-2000iV

The results of the more than 400 samples obtained with the Sysmex XT were compared to the results from the reference methods regarding clinical implications. These data are compiled in Table 7. For RBC and HGB, the discrepancies between the results from the Sysmex XT and the reference methods were only minor, and in no case would have led to different clinical conclusions. Different clinical conclusions were drawn in the following samples: out of 409 samples, a total of 26 showed deviations from the reference method for WBC, which would have led to a different clinical interpretation. Of these 26, the deviations were minor in 20 samples and major in six samples. For LYM, 20 out of 27 samples with deviations were major, for MONO 31 out of 35, for NEU 5 out of 9, for EOS 28 out of 41, for MCV 2 out of 82, for HCT 3 out of 46, for RET 28 out of 30, for IRF 25 out of 28, and for PLT 7 out of 14 samples with deviations. In addition, the Sysmex XT detected low monocyte counts wheras these cells were not seen in the blood smears.

Table 7 clinical relevance of the the Sysmex results that deviate from those of the reference methods

Parameter	n total	Correctly recognized samples/evaluated samples			not correctly recognized samples		
		<reference range	Within reference range	>reference range	n total	minor deviations	major deviations
RBC	409	117/120	264/268	20/21	8	8	-
HGB	409	185/1:8	211/215	6/6	7	7	-
HCT	409	172/185	185/219	5/5	46	43	3
MCV	409	144/144	166/244	17/21	82	80	2
MCHC	409	41/79	192/317	7/13	169	159	10
RET #	100	-	33/61	37/39	30	2	28
RET %	100	-	22/49	50/51	28	5	23
IRF #	100	-	61/61	11/39	28	3	25
IRF %	100	-	49/49	24/51	27	0	27
PLT	100	44/46	42/53	0/1	14	7	7
WBC	409	23/28	236/255	124/127	26	20	6
LYM	193	43/62	118/126	5/5	27	7	20
MONO	201	0/11	136/159	30/31	35	4	31
NEU	193	8/11	116/118	60/64	9	4	5
EOS	208	42/61	96/109	29/38	41	13	28

3.6 Message codes

The different IP messages that were observed during the analyses of the 409 samples are shown in Table 8. 379 out of 409 blood samples analyzed displayed one or more message codes. A total of 49 samples generated the message code “abnormal white blood cell scattergram,” because of difficulties in assigning cells to a certain population. The blood smears of these samples showed that, in 19 cases, a left shift of the neutrophils was present, while eleven others contained aggregated platelets or giant platelets. Three samples were derived from cats with tumors (lymphomas due to infection with FeLV), where tumor cells, that could not be recognized correctly, appeared in the blood. For the remaining samples (n=16), no obvious errors could be found. The message code “abnormal distribution of platelets” was displayed because of a failure to differentiate between platelets and RBC for a portion of cells. Of the 36 samples that did not show this message code, 19 were below, 15 within, and two above the reference value for PLT. One sample with 500,000 reticulocytes and 4.5 $x10^6$ erythrocytes was marked with an “abnormal reticulocyte scattergram,” because the instrument was unable to differentiate erythrocytes from mature reticulocytes. Two samples, which came from the same cat on two consecutive days, showed the message code “error,” but no explanation was provided.

Table 8 message codes from the Sysmex XT

Message code	Flagged samples: 379 out of 409	Message code confirmed by observation
WBC abnormal scattergram	49	19 with left shift; 11 with aggregates of PLT or giant PLT; 3 with tumor cells
PLT abnormal distribution	373	because PLT are almost the same size as RBC
RET abnormal scattergram	1	500'000 RET at 4.5 x10^6 RBC, could not distinguish RET from RBC
Error	2	The same cat on two days in a row

3.7 Aging of the cells

The parameters WBC, MCH, and PLT-O remained unchanged during the 24-hour analysis period. The parameters RBC, HCT, HGB, MCV, NEU, EOS, and RET increased during the 24 hours of the study, while MCHC, LYM, MONO, and PLT-I decreased. These data are compiled in Table 9.

Table 9 Aging of the cells

Parameter	Effect	Percentage [a]	Significance [b]	Time span during which no significant deviations from freshly measured values were observed [c]
WBC	None	None	0.3787	24
RBC	Increase	2.1	0.1677	24
HGB	Increase	1.4	0.019	24
HCT	Increase	17.6	<0.0001	5
MCV	Increase	15.2	<0.0001	7
MCH	None	None	0.0089	24
MCHC	Decrease	13.7	<0.0001	7
PLT-O	None	None	<0.0001	24
NEU	Increase	7.1	0.4099	24
LYM	Decrease	13.3	0.1418	8
MONO	Decrease	7.7	0.0235	24
EOS	Increase	12.1	0.0308	24
RET#	Increase	41	<0.0001	10
PLT-I	Decrease	8.8	<0.0001	24

[a] of change within 24 hours at 25° C
[b] of deviation from time of collection within 24 hours at 25° C (paired t-test, which compares each measurement with t =0)
[c] < 5% deviation from time of collection

4 Discussion

4.1 General performance of the Sysmex XT-2000iV

The Sysmex XT-2000iV was found to be a well-designed and extremely user-friendly instrument, which was very easy to handle and could be operated after just a small amount of instruction. The analysis time per blood sample was around one minute, which would allow this hematology instrument to be used for analysis of a large number of samples under routine service conditions. Also, maintenance, as well as the restocking of reagents, was remarkably fast and easy. The overall reliability was remarkable, as the instrument did not present any technical difficulties during the evaluation time of nine months.

4.2 Precision

CVs within series of up to 3% are usually considered good, and CVs of up to 5% are acceptable for cell counts (Bollinger, Drewinko *et al*. 1987; Winkler *et al*. 1995; Wegmann, Hofmann-Lehmann *et al*. 1997). For statistical reasons, CVs tend to be higher if the number of counted cells is small. Good precision was achieved for the measurements of all RBC parameters, with the exception of reticulocytes, which had CVs of 6.8% for high, and 6.4% for low, RET. It was expected that, due to statistical reasons, the CV of high RET would be lower than that for samples with low RET. No explanation can be offered for why this did not occur. However, the precision in series of the Sysmex XT for RET was found to be far better than that for the manual count. The platelets measured by the optical method showed good to acceptable precision, depending on the amount of PLT present in the sample. On the other hand, platelets measured with the impedance method had good precision in samples with high numbers of platelets, but bad precision for those with lower numbers. The precision of the measurements of WBC, NEU, LYM (except for high numbers, which were moderate), and MONO (except for low numbers, which had a CV of 21%) were good to acceptable. The precision measurements for EOS at all three levels tested, and for BASO at high numbers of BASO, were moderate. However, at the average level, the CV was considered high, at 26%. The rather high CVs of EOS and BASO can be explained by the low mean values, and cannot be attributed solely to the measurement system. Therefore, the precision of the instrument for the measurement of these cells cannot be specified.

One advantage of an electronic cell counter over manual differentials is the high number of cells counted during each measurement. This results in a much better statistical distribution and higher measurement precision than is seen with any microscopic method (Pohland 1989).

For the determination of the precision from day to day, we used trilevel control blood provided by the manufacturer. The observed CVs for WBC, RBC, HGB, HCT, MCV, MCH, and MCHC were good, for PLT, NEU, BASO, and RET acceptable, and for LYM, MONO, and EOS moderate. Generally, the low control blood sample exhibited higher CVs than the normal or high control samples, which was expected for statistical reasons.

4.3 Linearity of the measurement range

Given that all measured parameters showed no deviation of linearity over the tested range, the linearity was considered to be excellent. The linearity of monocytes and basophils could not be calculated due to the very low numbers of cells present in the samples evaluated.

4.4 Accuracy

Some of the parameters differed significantly from those of the reference methods. Although these differences were statistically significant, they were not of clinical importance. The correlation coefficient (r) is a measure of the comparability of measurement methods. Under linear conditions, an r with a value of 1 or -1 reflects total equality between the results of both methods. An r >0.95 can be rated as very good, and an r >0.8 as acceptable (Bollinger, Drewinko *et al*. 1987; Tvedten and Wilkins 1988). In addition to the determination of r, the intercept and the slope must be considered (Tvedten and Korcal 1996). If the intercept and the slope deviate from 0 and 1, respectively, systematic errors have to be assumed. To characterize the accuracy of the Sysmex XT, we not only evaluated the correlation coefficient, but also the intercept and the slope. Good to very good correlation between the values of the Sysmex XT 2000iV and those of the reference methods was observed for WBC, NEU, LYM, RBC, HGB, HCT, MCV, MCH and PLT. Acceptable correlation was seen for EOS and RET. The correlations for monocytes, basophils and MCHC were less satisfactory. For the monocytes, this was probably attributable to the "cloud phenomenon," which occurred in several different samples that had nearly the same counts. For the basophils, the low precision and the lack of a sufficient number of samples with significant counts of this cell type could explain the insufficient accuracy. In the case of MCHC, the bad correlation was also due to the cloud phenomenon. The counting of PLT is often complicated by the fact that they cluster together. This clustering is most likely due to the blood collection, where the endothelium of the thin vessel is punctured by the needle, causing the coagulation cascade to be initiated. In such cases, PLT counts cannot be determined exactly, but only estimated. In our study, in 31 of 131 samples, the PLT were present in clusters, and these samples had to be excluded from statistic analysis.

4.5 Representation of the measured values

A strong association was found between a left shift of the neutrophil granulocytes and the occurrence of a shifted cloud of neutrophils in the scattergram to the upper left, or even a fusion with the clouds of lymphocytes or monocytes. Seventeen out of 70 samples with a left shift showed fused clouds, and the Sysmex XT was not able to differentiate between those populations; 39 out of the 70 samples showed fused clouds, but a differentiation was made. In the other 14 samples, no distinctive feature was seen in the scattergram. Given these results, it is recommended that samples showing a shift of neutrophils to the upper left of the dot plot, especially those that have fused clouds, be microscopically differentiated.
The scattergram of a blood sample from a cat that received a blood transfusion one day prior to blood collection clearly showed two populations of RBC and RET, a smaller

population representing the cat's own cells and a larger population derived from the donor cat.

4.6 Evaluation of the clinical relevance of the results obtained with the Sysmex XT-2000iV

The vast majority of the results obtained with the Sysmex XT-2000iV would have led to similar clinical interpretations as the results obtained using the reference methods. In the few cases, where different clinical conclusions would have been drawn, the discrepancies can mainly be attributed to the high imprecision of the microscopic evaluation of the blood smears. The fact that the Sysmex XT did not recognize 11 samples with low monocyte counts was of no clinical relevance and could be explained by lack of detection of low monocyte counts in the blood smears. This could be explained by the fact that white blood cells, especially monocytes, are not evenly distributed on blood smears (Tvedten and Wilkins 1988), and that only 200 cells are counted manually, while the Sysmex XT instrument counts a multiple of that, which makes the instrument statistically better.

4.7 Message codes

Message codes, also called flags, were displayed when the Sysmex XT judged a sample to be abnormal regarding a particular parameter (abnormal cell population, particle size distribution, or scattergrams). The message code "WBC abnormal scattergram" was displayed when the instrument could not differentiate between cell populations. Of the 49 samples displaying this message code, 19 demonstrated a left shift of the neutrophils, and 11 contained aggregates of platelets. These abnormalities may explain why message codes were displayed in these cases. To ensure that these clinically important incidents are not missed, we recommend microscopically examining blood smears of samples displaying this flag. The "platelet abnormal distribution" message code was seen in 373 out of 409 samples. This is due to the fact that cats have large platelets, which are similar in size to small erythrocytes, making it nearly impossible to clearly distinguish between these two components of the blood. Nonetheless, 36 samples did not show this message code. Of these, 21 were either below or above, and 15 were within, the reference value.

4.8 Aging of the cells

Blood collection is associated with several pre-analytical variables, including the correct ratio of anticoagulant to blood volume, storage time in the anticoagulant, and temperature of the blood sample. The effect of aging on cat blood was determined over a period of up to 24 hours, during which the samples were stored at room temperature. Only three parameters (WBC, MCH and PLT-O) remained absolutely stable over the observation time. Five showed only little change, while six parameters showed significant increases or decreases. The fact that the HCT increased by 17.6% over 24 hours is important for laboratories receiving samples by mail or courier. Because of this phenomenon, an anemic patient may appear normal. We suspect that this occurs because the osmolarity of the Sysmex XT diluent is 250 mosm/kg, while the diluent of

the CellDyn is 312 mosm/kg. This hyperosmolarity is used to make the flat erythrocytes more spherical, because spherical erythrocytes are more easily counted. However, as erythrocytes age, they lose their osmotic resistance. This allows more fluid to enter the cell, and, as a consequence, the erythrocytes swell, and MCV and HCT increase. Also worth noting is the fact that RET increased over 24 hours by 41%. This effect could cause a non-regenerative anemia to appear as a regenerative anemia. Nevertheless, analysis of blood samples can be performed up to 8 hours after collection without significant loss of precision or accuracy.

4.9 Pathologic samples

During this study, a feline sample submitted to the laboratory generated a very high leukocyte number reading from the CellDyn 3500. Manual counting of a dilution of the sample, which was required to verify the CellDyn 3500 reading, demonstrated a tremendous number of 150,000 leukocytes per µl. This number was very close to the result generated by the Sysmex XT in the undiluted mode. This suggests that the Sysmex XT-2000iV is very reliable in identifying leukocytosis.

5 Conclusion

The Sysmex XT-2000iV was found to be very suitable for use with feline blood. The results of 31 hematological parameters are obtained, with little effort, within one minute. Results for WBC, NEU, LYM, EOS, RBC, HGB, HCT, MCV, MCH, PLT and RET showed a high degree of reliability. Quantitation of RET allowed characterization of regenerative versus non-regenerative anemias. Based on evaluation of the clinical implications of the results provided by the Sysmex XT, the user should be aware that, occasionally, lymphocytopenias or monocytopenias might be missed. In cases where no clear evaluation of a sample is possible, a message code will be displayed. The extension of neutrophils towards the lymphocyte population was found to be a reliable indicator of the presence of immature neutrophils in the blood sample, a fact that is very useful during clinical interpretation of a sample (Mathy and Koepke 1974). The ability to recognize abnormal cell morphologies and blood cell precursors is limited, as it is for all types of automatic cell counters. The message codes provide helpful indications as to which samples should be analyzed microscopically.

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